

# 1774 Calibration, measurements stability and replacement of standard cottons for an FMT3

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## ABSTRACT :

Daily calibration of the FMT3 machine is required to compensate the effects of sample preparation by different technicians and the ageing of the apparatus. As this calibration involves the use of a limited stock of standard reference cotton, the problem of replacing the standards arises when the latter are exhausted. The standards are described by two values called PL and PH. This study covers the stability of measurements in time, the incidence of two types of calibration, the validity of the corrections made by calibration and the evaluation of the PL and PH values of new standards by several technicians. The USDA ICCS standards used are very homogeneous and supplied in 225-g rolls. The differences between technicians and the heterogeneity of the rolls are taken into account using a randomized experimental design and a linear model. It is simple to use and requires little material and technician time. A daily calibration by regression ensures a greater stability of FMT3 measurements and compensates any technician effect.

**KEYWORDS:** maturity, FMT, calibration, stability, bias

**TITLE:** Calibration, measurements stability and replacement of standard cottons for an FMT318

## INTRODUCTION

Fibre maturity is an important feature as mature fibres absorb dye better and are less prone to cause defects of various sorts in the finished product. It is also important for breeders as it may show some defects in the suitability of a variety to its environment. The commonest method of measurement of micronaire (IM), fineness (H) and maturity (MR) is based on the analysis of the resistance to air flow (PL and PH) through cleaned cotton that a technician has placed in a short cylinder. These measurements are defined by Montalvo et al., 2002. Some models of the devices called "Fineness and Maturity Tester" (FMT) manufactured by SDL-Atlas, Stockport, England, have long been used by CIRAD for taking maturity characteristics into account in breeding programs.

Many factors can disturb the measurement of maturity by compression in an air flow: the condition of the apparatus and especially O-rings, various adjustments, laboratory conditions or preparation and handling of the sample by the technician (Montalvo et al., 2002). The apparatus must therefore be calibrated satisfactorily to give readings that are stable in time and identical from one machine to another. Reference or 'standard' cottons are required for this.

To the best of our knowledge, there is no recognised, commercially available international standard with PL and PH values to calibrate maturity evaluation apparatus (FMT® or Micromat®). USDA proposes "ICCS USDA" cottons recognized for their good homogeneity but that are only validated for micronaire value IM (USDA, 1989). For this reason, the manufacturer SDL-Atlas supplies with each FMT3 apparatus two standard cottons of ICCS origin and for which he has determined the PL and PH values. Each standard is taken from a single bale weighing some 225 kg and is supplied in half-pound

(225 g) rolls. As PL and PH measurement is destructive, each calibration requires two cotton test samples each weighing grams. The calibration 1 cotton runs out sooner or later and the parameters of the calibration software must be adjusted to match new standards. Unfortunately, the user cannot perform this operation. If a laboratory wishes to perform routine calibration of the apparatus as often as necessary—in our opinion at each change of technician and for each half-day of work—a substantial stock of calibration standard material must be acquired on purchase of the apparatus. If this has not been done, the laboratory faces the problem of performing calibration using its own resources if it is desired to ensure long-term stability of the maturity characteristics provided for clients. This problem encompasses the homogeneity of standard cottons, allowance for the possible effect of preparation by different technicians and finally the appropriateness and accuracy of the corrections made.

The methods that can be used to maintain a standard level by daily calibration and the way in which we replace one standard by another when the first is practically exhausted are described here. We check the appropriateness of calibration by use of a correction factor or regression and evaluate new standards by allowing for the differences between technicians and between rolls.

## **MATERIAL AND METHODS**

Samples were prepared using an SDL 099 fibre blender filled with 10 to 12 grams raw fibre, opened by hand and spread out in a layer about 40 cm by 10 cm.

All the cottons were analysed using an FMT (model 3) maturity tester (Shirley Developments, 1984) with no particular modifications. Air pressure at input was maintained at the required 6 bars. Adjusted for 1 L and 4 L per minute, a rotameter float allows accurate adjustment of the airflow used in each of the two depression measurements. This flow was checked during every daily maintenance operation. The two cottons supplied by the manufacturer are used to calibrate the apparatus before the first readings are performed on the samples to be evaluated.

### **Variables subjected to calibration**

Depressions PL and PH are the subject of calibration. However, the FMT model 3 does not display or transmit these variables directly, but rather micronaire value IM, maturity ratio MR, mature fibre percentage PM and fineness H (Lord & Heap, 1988):

$$H = \frac{60,000}{PL} \left( \frac{PH}{PL} \right)^{1.75}$$

$$IM = \left( \frac{850}{PL + 40} \right) + 0,6$$

These equations are reversed to obtain the PL and PH values to be corrected, giving:

$$PL = \left( \frac{850}{IM - 0.6} \right) - 40$$

$$PH = \left( \frac{PL^{2.75}}{60,000} \right)^{\frac{1}{1.75}}$$

## Cottons and technicians

### *Routine calibration using old standards*

This routine calibration does not replace the obligatory calibration executed by the software for the apparatus. However, this only takes place at each maintenance operation—consisting mainly of inspecting the piston O-ring and changing it if necessary. A cascade of two calibration operations is thus performed. The first is planned in the operation of the machine, but with a limited stock of cotton, and the second by us with a larger stock allowing everyday compensation for the various interfering effects: ageing of O-rings and of the machine between two maintenance operations, preparation and handling by the technician. Two methods of calibration are compared: the correction factor (F) and linear regression (R). The correction factor is estimated by the ratio of the reference measurement to the measurement of the day on the same standards. With the other procedure, the coefficients of the affine transformation are those of the linear regression of the reference measurement to the measurement of the day. We henceforth use the term 'observed result' for that given by the FMT3 machine and its own calibration system, and 'corrected result' for that further corrected by multiplicative correction (F) or by regression (R).

Three USDA ICCS cottons, C38, L01 and M01, covering a broad range, are used as reference and their PL and PH values form the level to be conserved. These are referred to as theoretical values and were determined in late 1998 using FMT model 1 (Shirley Developments, 1977), with 3 technicians each performing 20 measurements (10 pairs of 2 fibre samples) weighing  $4g \pm 0.05g$ . All the cottons to be tested were prepared with the same blender under the same conditions of temperature and relative humidity, 21°C and 65% RH (in conformity with the standard ISO 139).

The values of these three standards have been used on a routine basis since 2000 to calibrate the FMT3 using our own procedure. Before each half-day of work, a technician performs the calibration to be used for his measurements: he tests each of the 3 standards on two blended fibre samples weighing 3.8 to 4.2 g. The figures recorded on the FMT3 are entered in a SISTER® database (Gourlot & Giner, 1999) and used to correct the measurements before they are delivered to the client. Set up in May 2000 and maintained by the 9 technicians who have worked in the laboratory, it contains determinations of PL and PH for 1445 triplets of the 3 cottons C38, L01 and M01. This record makes it possible to check the stability of the raw measurements and, to a certain extent, the validity of the corrections, either with a correction factor (F) or a linear regression (R).

### *Evaluation of the new standards*

We conducted two experiments, each aimed at evaluating the PL and PH values of the USDA ICCS standards: first cotton C39 and then cotton L02.

Each new standard was from the same bale. It was evaluated by the 7 permanent technicians at the laboratory. As it is available in several 225 g rolls, each experiment combined several rolls and several technicians using a randomized plan (Tables 1 and 2) in order to evaluate the variations between rolls, and the second consisted of two separate replicates. Each replicate required no more than half a day's work per technician. Little standard material was used: the study required a total of 30 g per roll of cotton C39 and 60 g per roll of L02 cotton. Each technician performed two measurements on each valid specimen of between 3.8 and 4.2 g of fibre weighed accurately on an Ohaus balance (accuracy 0.01 gram). Each half-day was preceded by measurements on the usual C38, L01 and M01 standards to calculate the correction factors for that half-day.

### Statistical analysis

We first sought to verify whether one or other of the calibration methods that we propose is relevant. The values studied were the 'observed' figures given by the FMT3 with its own calibration. Then, to evaluate the PL and PH of the new standards, we used the 'corrected' figures, either by a correction factor (F) or a regression (R).

Statistical analyses and graphs were carried out using SAS/Stat and SAS/Graph software, version 9 (SAS Institute Inc., Cary, NC, USA), namely the GLM and MIXED procedures, and the GLIMMIX macro.

### *Validity of the correction factor*

A correction factor is sufficient if all the variations between cottons are proportional from one technician to another or, more generally, from one half-day of work to another. In routine calibration operations, this means that the regression equations should theoretically have a nil intercept. In practice, the estimated intercepts that can be obtained at each calibration operation, would then be distributed randomly around 0. This hypothesis was tested by a Student test on the sample of intercepts obtained by the calibrations performed by each technician.

In the experiments on the new standards, with the same hypothesis of proportionality, if  $i$  is the cotton index,  $j$  the technician index and  $k$  the roll index, the expected measured value should be:  $E(Y_{ijk}) = \mu \alpha_i \beta_k$ . On the logarithm scale,  $\text{Log}(E(Y_{ijk})) = m + a_i + b_k$ , the cotton and technician effects are additive, in other words there is no interaction. Absence of interaction can be tested with a generalised linear model (McCullagh and Nelder, 1983) in which additivity is tested on the log scale but errors are considered Gaussian in the original scale. To separate the random between-roll variations from measurement errors, the roll effect is added as an extra random term, making it a generalized linear mixed model.

### *Validity of the linear regression*

As calibration regressions are calculated at 3 points, the linearity of the relation is tested with verification that the residuals are nil on average for each cotton and more precisely for each cotton and each technician. This was performed using the following linear model in which  $i$ ,  $j$  and  $t$  are the indices for cotton, technician and time,  $Y_{ijt}$  is the measured value and  $x_i$  is the known value of cotton  $i$ :

$$Y_{ijt} = a_{jt} + b_{jt}x_i + c_i + d_{ij}$$

The first two terms are the regression equation varying according to the technician  $j$  and time  $t$  and the last two are the supplementary effects of the cotton and the interaction between cotton and technician; these two terms are nil if linear calibration is valid. This test is used both on routine calibration records and on experimental data, where index  $t$  is replaced by the replicate number. In the first experiment with only one replicate, term  $d_{ij}$  disappears

as it is confounded with error. We also compared the calibration values with the average values predicted by the calibration equations in order to check the practical importance of deviations from calibration.

### **Evaluation of new standards**

We analyse the values corrected by the two calibration methods; both are assumed to follow a linear mixed model, with roll effects and technician replicate effects (two replicates per technician) being variance components. The replicate effect encompasses the calibration error, only for the new cotton that has not contributed to calibration. The new standards are evaluated by their average values for all the technicians, each having equal weighting whatever the number of rolls he or she processed. Standard errors are estimated according to the variance components and experimental error. As replicates exist only for the L02 new standard, standard errors are not calculated for the C39 cotton.

## **RESULTS and DISCUSSION**

### **Routine calibration of old standards**

#### ***Stability and variability in time before and after correction: graphic description***

The observed results presented in chronological order in Figures 1a and 2a display fairly large dispersion of PL and PH, accentuated by rounding problems for PL. Indeed, the values electronically transmitted by the FMT3 are rounded off to one significant digit less than in the paper version. This results in horizontal alignments of points and visibly has a marked impact on the accuracy of the PL results. In contrast, PH was not visibly affected. A slight decrease in PH was observed in the early months while the larger dispersion of PL results meant that this could not be detected. The decrease in PH was confirmed by linear regression according to the sequence number of the calibration operation (Table 3).

The simple correction factor stabilises the measurements considerably (Figs. 1b and 2b). Further improvement can be achieved with correction by regression (Figs. 1c and 2c).

#### **Validity of correction by multiplicative factor and by linear regression**

If correction by multiplicative factor were sufficient, the calibration lines would theoretically pass via 0. It is seen in Table 4 that the average of the intercepts is significantly different from zero for each technician. Student  $t$  values are all significant with one exception. Calibration by correction factor is therefore biased.

For regression, the non-linearity 1 test described in the Material and Methods section is also significant (Table 5): there is consistent departure from linearity in all the regressions and the relation between observed values and theoretical values is not linear in fact. Thus, in theory, neither correction by multiplicative factor nor that by regression is satisfactory as both generate biases in the average values with either method. However, comparison of the theoretical values with the average values obtained shows that these deviations are not of great practical importance (Table 6).

These results that are stable in time at CIRAD do not reveal any noteworthy ageing of the standard or of the machine and show that the calibration of a new standard using old ones is legitimate.

**Evaluation of new standards** The validity of correction by multiplicative factor was tested using the technician x cotton interaction on the log scale (Table 7). It was rejected for cotton L02, but not for C39. The linearity of the relation between theoretical value and observed value in the known standard was tested by average deviation by cotton (cotton effect) and the variations in these deviations according to technician (tech\*cotton interaction). The only significant figures were the average deviations by cotton for the PL variable in the second experiment (Table 8). The bias of the correction by multiplicative factor is more evident than that of the correction by regression. With both calibration methods, the figures recorded as the new reference for the laboratory are shown in Table 9. The figures corrected by regression show more variability than those corrected by multiplicative factor.

## DISCUSSION-CONCLUSION

Measurements using FMT3 apparatus require calibration to cancel drift, reduce variability and compensate the effect of the preparation and handling of the apparatus by different technicians.

As calibration by regression or by multiplicative factor gives stable measurements in time, changes of standard can be envisaged with each new one calibrated with its predecessors. We needed to develop a simple, rapid and effective technique to determine them with sufficient accuracy.

The choice between the two calibration methods is not made: while the linear regression method is less biased than the constant factor method, its results are more variable on the cotton that has not participated to the calibration. The compromise between bias and variance has yet to be determined.

A shift, even limited, is inevitable when a change is made as zero error cannot be guaranteed at a change in standard. Inter-laboratory maturity tests are therefore required so that laboratories can calibrate between each other and avoid individual long-term drift.

Calibration software should be modified so that standards can be changed, to avoid a routine cascade of two calibration operations. Furthermore, it would be preferable to base calibration on three cottons rather than two, with a warning given in case of a sizeable deviation from linearity.

Although it requires a temporary increase in daily calibration work, the overlapping of successive standards would be another way of achieving a smooth transition from one standard to another. It would also allow the empirical comparison of calibration accuracy by constant factor and by regression.

The micromat operates using 1 g the same principle as the FMT3 and was tested in a fairly similar experiment (Gawrysiak et al., 1998). The data should be used in the same way to extend these results to other apparatus.

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## TABLES

Table 1: Experimental design of study 1: 25 rolls of ICCS C39, one replicate.

| Roll number        | Technicians |   |   |   |   |   |   |
|--------------------|-------------|---|---|---|---|---|---|
|                    | 1           | 2 | 3 | 4 | 5 | 6 | 7 |
| 01, 04, 09, 15, 25 | X           | X |   |   |   |   | X |
| 06, 10, 13, 19, 24 | X           |   | X |   |   |   | X |
| 03, 08, 11, 18, 20 | X           |   |   | X |   |   | X |
| 05, 12, 14, 17, 23 | X           |   |   |   | X |   | X |
| 02, 07, 16, 21, 22 | X           |   |   |   |   | X | X |

Table 2: Experimental design of study 2: 20 rolls of ICCS L02, two replicates.

| Roll number    | Replicate   |   |   |   |   |   |   |             |   |   |   |   |   |   |
|----------------|-------------|---|---|---|---|---|---|-------------|---|---|---|---|---|---|
|                | A           |   |   |   |   |   |   | B           |   |   |   |   |   |   |
|                | Technicians |   |   |   |   |   |   | Technicians |   |   |   |   |   |   |
|                | 1           | 2 | 3 | 4 | 5 | 6 | 7 | 1           | 2 | 3 | 4 | 5 | 6 | 7 |
| 01, 04, 09, 15 | X           | X |   |   |   |   | X | X           | X |   |   |   |   | X |
| 02, 07, 16, 20 | X           |   | X |   |   |   | X | X           |   | X |   |   |   | X |
| 03, 08, 11, 18 | X           |   |   | X |   |   | X | X           |   |   | X |   |   | X |
| 05, 12, 14, 17 | X           |   |   |   | X |   | X | X           |   |   |   | X |   | X |
| 06, 10, 13, 19 | X           |   |   |   |   | X | X | X           |   |   |   |   | X | X |



Table 3: Long-term trends in raw measurements: regression of observed values of PL and PH against the sequence number of the calibration operation.

| Depression | Cottons | Equations                            | P value of test of  slope  ≠ 0 |
|------------|---------|--------------------------------------|--------------------------------|
| PL         | L01     | $PL = +84 \cdot 10^{-06} X + 175.3$  | 0.4984                         |
|            | C38     | $PL = -116 \cdot 10^{-06} X + 241.4$ | 0.4775                         |
|            | M01     | $PL = -205 \cdot 10^{-06} X + 310.0$ | 0.3748                         |
| PH         | L01     | $PH = -585 \cdot 10^{-06} X + 129.6$ | <0.0001                        |
|            | C38     | $PH = -588 \cdot 10^{-06} X + 186.1$ | 0.0003                         |
|            | M01     | $PH = -973 \cdot 10^{-06} X + 255.2$ | <0.0001                        |

Table 4: Regressions of the observed PH and PL on their theoretical values at each routine calibration: test of 0-mean of the intercepts.

| Technician | n   | Dependent variable |           |       |       |           |       |
|------------|-----|--------------------|-----------|-------|-------|-----------|-------|
|            |     | PL                 |           |       | PH    |           |       |
|            |     | Mean               | Std Error | t     | Mean  | Std Error | t     |
| 1          | 80  | 13.63              | 1.28      | 10.65 | 5.88  | 1.49      | 3.95  |
| 2          | 56  | 14.45              | 1.96      | 7.37  | 4.38  | 1.86      | 2.35  |
| 3          | 168 | 17.77              | 0.92      | 19.32 | 7.08  | 0.89      | 7.96  |
| 4          | 408 | 12.37              | 0.48      | 25.77 | 2.17  | 0.48      | 4.52  |
| 5          | 37  | 16.74              | 2.09      | 8.01  | 7.44  | 1.82      | 4.09  |
| 6          | 260 | 14.77              | 0.87      | 16.98 | 4.76  | 0.82      | 5.80  |
| 7          | 51  | 15.81              | 1.39      | 11.37 | 5.24  | 1.21      | 4.33  |
| 8          | 237 | 16.03              | 0.76      | 21.09 | 6.50  | 0.70      | 9.29  |
| 9          | 56  | 9.87               | 1.45      | 6.81  | -1.85 | 1.48      | -1.25 |

Table 5: Regressions of the observed PH and PL on their theoretical values at each routine calibration: test of departures from linearity (cotton effect) and of dependance of these departures on the technician (tech x cotton interaction)

| Depression | Source        | DF | Type I SS | Mean Square | F Value | Pr > F |
|------------|---------------|----|-----------|-------------|---------|--------|
| PL         | cotton        | 1  | 20348.25  | 20348.25    | 658.53  | <.0001 |
|            | tech x cotton | 8  | 732.90    | 91.61       | 2.96    | 0.0026 |
| PH         | cotton        | 1  | 16836.05  | 16836.05    | 428.42  | <.0001 |
|            | tech x cotton | 8  | 694.81    | 86.85       | 2.21    | 0.0240 |

Table 6: Routine calibration: comparison of theoretical and mean observed values and those obtained using a multiplicative factor (F-Corrected) and regression (R-Corrected) for PL and PH. (Nb) is the number of calibrations achieved with each cotton.

| Cottons (Nb) | Theoretical |       | Observed |       | F-Corrected |       | R-Corrected |       |
|--------------|-------------|-------|----------|-------|-------------|-------|-------------|-------|
|              | PL          | PH    | PL       | PH    | PL          | PH    | PL          | PH    |
| L01 (1442)   | 176.2       | 136.7 | 175.5    | 128.3 | 178.6       | 136.5 | 174.7       | 135.2 |
| C38 (1454)   | 241.9       | 193.3 | 241.1    | 184.8 | 245.3       | 196.5 | 245.4       | 196.6 |
| M01 (1440)   | 320.9       | 272.1 | 309.6    | 253.1 | 314.9       | 269.0 | 318.9       | 270.3 |
| Mean (4338)  | 246.3       | 200.7 | 242.1    | 188.7 | 246.3       | 200.6 | 246.3       | 200.7 |

Table 7: Test of the validity of calibration by correction factor: technician x cotton interaction on the log scale indicates that this correction is biased.

| Type 3 Tests of operator x cotton interaction |     |        |         |         |        |
|---|-----|--------|---------|---------|--------|
|   |     | Num DF | Den DDL | F Value | Pr > F |
| PL  | C39 | 18     | 37.6    | 1.64    | 0.0990 |
|   | L02 | 18     | 119.0   | 3.59    | <.0001 |
| PH  | C39 | 18     | 44.6    | 1.08    | 0.3981 |
|   | L02 | 18     | 121.0   | 3.77    | <.0001 |

**Table 8: Evaluation of new standards C39 and L02: test of departures from linearity when regressing observed measurements of old standards on their known values.**

| Cotton | Depression | Tested effect | DF | Type I SS | Mean Square | F Value | Pr > F |
|--------|------------|---------------|----|-----------|-------------|---------|--------|
| C39    | PL         | departure     | 1  | 29.94     | 29.94       | 0.69    | 0.4277 |
|        | PH         | departure     | 1  | 62.78     | 62.78       | 1.02    | 0.3391 |
| L02    | PL         | departure     | 1  | 123.41    | 123.41      | 5.43    | 0.0366 |
|        |            | tech. x dep.  | 6  | 93.86     | 15.64       | 0.69    | 0.6632 |
|        | PH         | departure     | 1  | 59.42     | 59.42       | 3.07    | 0.1035 |
|        |            | tech. x dep.  | 6  | 164.68    | 27.45       | 1.42    | 0.2805 |

**Table 9: Estimated PL and PH values for the new standards C39 and L02, after calibration with multiplicative factor (F) or regression (R).**

| Cotton | Depression | F-Corrected |           | R-Corrected |           |
|--------|------------|-------------|-----------|-------------|-----------|
|        |            | Mean        | Std error | Mean        | Std error |
| C39    | PL         | 274.66      |           | 277.07      |           |
|        | PH         | 220.95      |           | 221.57      |           |
| L02    | PL         | 183.28      | 0.79      | 177.60      | 1.22      |
|        | PH         | 129.26      | 0.82      | 127.06      | 1.33      |

Figure 1: Evolution in time of PL in standard cottons L01, C38 and M01: observed values (a), values corrected by correction factor (b) and by regression (c). One different color for each technician.

Figure 2: Evolution in time of PH in standard cottons L01, C38 and M01: observed values (a), values corrected by correction factor (b) and by regression (c). One different color for each technician. ACKNOWLEDGEMENT: The authors thank researchers and technicians at the CIRAD cotton laboratory for FMT measurements and other contributions to this paper. DISCLAIMER: Mention of a trademark, warranty, proprietary product or vendor does not constitute a guarantee by CIRAD and does not imply approval or recommendation of the product to the exclusion of others that may be suitable. ABBREVIATION: Fiber Maturity Tester, model 3 (FMT3)



